

DESCRIPTION

AGARICUS EXTRACT FOR PREVENTING THE INDUCTION OR METASTASIS OF CANCER

5

TECHNICAL FIELD

The present invention is directed to an *Agaricus blazei* Murill extract comprising an ingredient being capable of preventing the induction or metastasis of cancer. More specifically, the present invention is directed to an *Agaricus blazei* Murill extract for preventing the induction of cancer by urethane (ethyl carbamate, EC) existing in fermented foods and alcoholic beverages, carcinogen (4-N-methyl-N-nitroamino)-1-(3-pyridyl)-1-buthanone (NNK) existing in tobacco smoke or azoxymethane (AOM), or the metastasis of the cancer induced by urethane, NNK or AOM.

BACKGROUND ART

Urethane and NNK are known to induce a variety of cancers. Urethane has induced lung cancer and liver cancer in mouse in a dose dependent manner, and the safety dose (virtually safe dose, VSD) thereof in human have been calculated to be 1.8×10^{-4} mg/kg body weight for lung cancer, and 7.2×10^{-5} mg/kg body weight for liver cancer, respectively (K. Inai et.al, Jpn. J. Cancer Res., 82, pp.380-385, April, 1991).

Progeny of the mouse treated by urethane during pregnancy and lactation period have developed significantly increased embryotic cancer, lung cancer and ovarian cystadenoma through placenta, and parent pregnant mouse also have developed endometrial hyperplasia and uterus hemangioma (T. Nomura, Cancer Research, 33, 1677-1683, July, 1973).

Orally administered urethane has induced lung cancer, lymphoma, liver cancer, gastric papilloma, sebaceous gland tumor, breast cancer, lung adenoma, tumor of squamous
5 epithelium cell, leukemia, mesenchymoma, and the like (IARC MONOGRAPHS ON THE EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO MAN, vol.7, pp.111-131, the views of two IARC Working Groups on the Evaluation of the Carcinogenic Risks of Chemicals to Man which met in Lyon, February 4, to 11,
10 1974; and June 18 to 24, 1974).

Further, urethane is an object of regulation in Canada, because it is produced in alcohol fermentation and contained at a predetermined level in a brewed beverage such as wine
15 (Ethyl Carbamate in Alcoholic Beverages and Fermented Foods, ACS Symposium Series No.484, Food Safety Assessment, edited by John W. Finley et al., pp.419-428, American Chemical Society, 1992; Identification of Volatile Constituents from Grapes, J. Agric. Food Chem., vol.24, no.2, pp.329-331, 1976;
20 RATIONALE FOR THE ESTABLISHMENT OF GUIDELINES TO LIMIT ETHYL CARBAMATE LEVELS IN ALCOHOLIC BEVERAGES, BUREAU OF CHEMICAL SAFETY FOOD DIRECTORATE HEALTH PROTECTION BRANCH, HEALTH & WELFARE CANADA, pp.1-8; Ethylcarbamate in Fermented Beverages and Foods, Cornelius S. Ough, J. Agric. Food
25 Chem., vol.24, No.2, pp.323-327.

NNK is one of the possible carcinogenic substances contained in a tobacco smoke (Djordijevic, M.V.et al., A Comparison of selected components in the mainstream smoke
30 of the leading U.S. and Japanese cigarettes. In: Proceedings of the CORESTA smoke and Technology Meeting, pp.200-217, November 3 to 8, 1996). The morbidity of lung cancer is increasing significantly in both males and females.

The incidence of lung cancer is highly associated with smoking and alcohol ingestion. The International agency for Research on Cancer suggests that the amount of smoking and
5 the amount of alcohol ingestion tend to increase synergistically in human, and that NNK and urethane ingested thereby are causes of lung cancer incidence.

Lung cancer accounts for 45% and 21.5% of cancer related
10 deaths in males and females, respectively. Currently, lung cancer is becoming a leading cause of death in comparison to heart disease among those who smoke in America.

Accordingly, food ingredients, which prevent the
15 induction of cancer caused by smoking and alcohol ingestion, have been sought.

The mushroom, which is generally called agaricus, belongs to the family *Agaricaceae* of the division *Basidiomycota*,
20 and is referred to by the botanical name "*Agaricus blazei* Murill" and the Japanese name "kawariharatake". Agaricus (hereinafter, generally referred to as kawariharatake, ABM, or agaricus) has been traditionally used as a medicament in the Piedade region in Sao Paulo, Brazil. It is said that
25 agaricus has a variety of immune activation activities, cancer prevention effects, tumor growth suppression effects, and the like. Currently, it is provided for internal use as health food.

30 Polysaccharides contained in agaricus include β -1, 6-glucopyranosyl residues and have antitumor activity against Sarcoma 180 (Ebina T et al. (1986), Jpn. J. Cancer Res 77:1034-1042). Extracts from agaricus include

(1→4)- α -D-glucan having (1→6)- β branched chain, and have natural killer cell activation activity and selective antitumor activity mediated through apoptosis (Fujimiya Y et al. (1998), Cancer Immunol Immunother 46:147-159).
5 Peptideglycans from agaricus have a direct cytotoxic activity against Meth A tumor cells in a double implanted tumor system and an indirect immune enhancement activity in tumor-bearing mice (Ebina T et al. (1998), Biotherapy 11:259-265). Polysaccharides contained in agaricus change the percentage
10 of spleen Thy1,2-, L3T4 positive cells in a T cell subset of mice (Mizuno M et al. (1998), Biosci. Biotechnol. Biochem. 62:434-437).

These reports suggest that polysaccharides contained
15 in agaricus have cytotoxic activity against tumor cells through an immunomodulation activity.

Thus, there have been many reports that say agaricus
20 extracts have an immune enhancement activity and antitumor activity. However, in order to test the effects of agaricus extract, previous researchers have used in vitro tests or in vivo tests with animals which already have cancer. Accordingly, the effects of agaricus extracts on processes of induction and metastasis of cancer such as lung cancer,
25 large intestine cancer, which are caused by a habit of living such as smoking or alcohol ingestion, have not been studied. It has not been reported that there is a food ingredient having effects on processes of inducing cancer and metastasizing cancer, to the best of inventor's knowledge.
30 It is considered that this is because there is no reliable assay system for evaluating processes of inducing cancer and metastasis of cancer.

DISCLOSURE OF THE INVENTION

Materials for drugs or foodstuffs capable of potentially preventing the induction or metastasis of cancer are provided.

5 The present invention is directed to an agaricus extract or an ingredient contained therein which is capable of preventing the induction (incidence) or the metastasis thereafter of cancer. The inventor has completed the present invention by finding that an agaricus extract or an ingredient
10 contained therein suppresses the genes or gene products which are overexpressed in A/J mouse being in the course of lung cancer induction by administering a carcinogenic substance, urethane or NNK, and by finding that an agaricus extract or an ingredient contained therein suppresses the formation
15 of aberrant crypt foci (ACF) in rat being in the course of large intestine cancer induction by administering a carcinogenic substance, AOM.

20 Although it is not desired to be constrained by a particular theory, it is considered that agaricus extract prevents the induction and the metastasis thereafter of cancer by containing an effective ingredient or ingredients which inhibit the activation of a carcinogenic substance, or remove the possible free radical produced by the metabolism of a
25 carcinogenic substance, or remove the free radical producer in a carcinogenic substance, or suppress the expression of oncogenes, or synergistic effects therebetween.

30 The present invention provides agaricus extract comprising an ingredient which is capable of preventing the induction and metastasis of cancer. According to one aspect of the present invention, the extract is prepared by extracting agaricus with a solvent.

The term "agaricus" used herein generally refers to agaricus materials comprising a fruit body, a mycelium, a culture broth and the like of a mushroom belonging to the family Agaricaceae of the division Basidiomycota, referred to by the botanical name "*Agaricus blazei* Murill" and the Japanese name "kawariharatake". The term "agaricus" typically means a fruit body of agaricus. Hereinafter, "kawariharatake", "ABM", and "agaricus" are used interchangeably, and they are terms indicating an identical agaricus material.

Preferably, the extract is prepared by extracting agaricus with hot water.

Preferably, the ingredient is a chromatographic main elute fraction of 100 to 2000 molecular weight obtained by the steps of extracting the (fruit body of) agaricus with hot water, dialyzing the resultant extract, and subjecting the thus obtained dialysis external fluid to chromatography.

Preferably, the ingredient is a dialysis external fluid obtained by the steps of extracting the fruit body of agaricus with hot water, adding ethanol to the resultant extract to thereby obtain precipitates, dissolving the precipitates in water, and dialyzing the solution.

The cancer can be those induced by urethane, (4-N-methyl-N-nitroamino)-1-(3-pyridyl)-1-butanone or azoxymethane. Typically, the cancer is lung cancer or large intestine cancer.

The present invention is also directed to a composition

being capable of preventing the induction or metastasis, comprising an agaricus extract, and a pharmaceutically acceptable carrier.

5 The composition can be in a form selected from the group consisting of powder, liquid, tablet, capsule and pellet.

BRIEF DESCRIPTION OF THE DRAWINGS

10 Figure 1 shows that an agaricus extract of the present invention prevents the induction of lung cancer caused by NNK.

 Figure 2 shows that an agaricus extract of the present invention prevents the induction of lung cancer caused by NNK in a dose dependent manner.

15 Figure 3 shows that an agaricus extract of the present invention suppresses a gene or a gene product induced by administering NNK.

 Figure 4 shows that an agaricus extract of the present invention suppresses a gene or a gene product induced by administering NNK in a dose dependent manner.

20 Figure 5 shows that an agaricus extract of the present invention prevents the induction of lung cancer caused by urethane.

 Figure 6 shows that an agaricus extract of the present invention suppresses a gene or a gene product induced by administering urethane.

 Figure 7 shows that an agaricus extract of the present invention prevents the induction of large intestine cancer caused by AOM.

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BEST MODE FOR CARRYING OUT THE INVENTION

 Agaricus extract of the present invention is prepared by extracting agaricus material with a solvent. The agaricus

material is typically a naturally occurring or cultured fruit body of agaricus. Cultured mycelium of agaricus in a culture tank or the like may also be used. Normally, agaricus is used after washing followed by drying. Commercially available dried fruit body is also used conveniently. Normally, dried agaricus is pulverized according to a standard method, and used as a starting material for extraction.

The agaricus extract of the present invention can be obtained by conducting an extraction procedure after adding a solvent to the dried fruit body or a powder thereof. In general, the dried fruit body, or the powder thereof, is mixed with a solvent of 2 to 10 times the weight thereof to conduct extraction. As the solvent, water, ethanol, propanol, butanol, acetone, 1,3-butylene glycol, ethyl acetate, hexane, methylene chloride, methanol, or a mixture thereof is used. Typically, water is used to prepare an agaricus extract.

Extraction procedure is conducted by mixing the agaricus powder with any of the above described solvents, and stirring or shaking with a magnetic stirrer (100 to 500 rpm) and the like, the thus obtained mixture, at a temperature between 0 to 100°C, preferably at a temperature between room temperature to 80°C, for a period of between 10 minutes to several days, preferably between 1 to 24 hours. Typically, the agaricus extract can be obtained by adding deionized water to the agaricus powder, and continuously stirring for 24 hours at 70°C. The thus obtained solution is lyophilized after removing the residue by a standard method such as centrifugation, filtration, and the like. The thus obtained powder is an agaricus extract.

The ingredient or ingredients capable of preventing the induction or metastasis being contained in the thus prepared agaricus extract can be obtained by utilizing a method known to those skilled in the art such as HPLC (high performance liquid chromatography), and the like. The structure of the obtained ingredient can be identified using a technique such as NMR (nuclear magnetic resonance), and the like.

The following is an example of a preparation method for an agaricus extract of the present invention.

Water with 5 to 10 times the weight of dried fruit body is added, and followed by heat-extracting or heat-refluxing the mixture for 1 to 3 hours. This hot water extraction from agaricus may be performed using a residue previously extracted with hot water. The solution extracted with hot water thus obtained is dried by a method known to those skilled in the art such as lyophilization, spray-drying, or the like to obtain a dried product (hereinafter, referred to as dried product A). Dried product A is mixed with 5 to 20 times the weight thereof of water. Then, the solution is put into a dialysis tube and dialyzed for 10 to 15 hours with several times the amount thereof of distilled water. The obtained dialyzate (external fluid) is lyophilized to obtain a dried product (hereinafter, referred to as dried product C) containing an ingredient or ingredients which are capable of preventing the induction or metastasis of cancer.

Then, the solution remaining in the dialysis tube is further dialyzed against running water for 20 to 40 hours and dialyzed twice against a distilled water for a few hours each time and a dried product of the solution remaining in the dialysis tube is obtained as described above. Thus, the

dried product (hereinafter, referred to as dried product B) containing any ingredient or ingredients which are capable of preventing the induction or metastasis of cancer can be obtained.

5

Next, the obtained dried product C is dissolved in about ten times the weight thereof of distilled water. Gel chromatography is performed with distilled water as an eluent to obtain 20 mL of fractions. From the obtained fractions, 10 a fraction or fractions in the middle of the elution peak, which has a molecular weight of about 100-2000 Da by gel filtration, is an ingredient or ingredients which are capable of preventing the induction or metastasis of cancer of the present invention.

15

These fractions were analyzed further using reverse-phase chromatography, which uses ODS (octadecyl silanated silica gel), ion-exchange chromatography using DEAE-TOYOPEARL 650, or the like, and confirmed to include 20 a plurality of ingredients such as arginine, lysine, mannitol, and the like.

The solution extracted with hot water, obtained by the above-described method, is mixed with an equal amount of 25 ethanol. The mixture is centrifuged to separate a precipitate from a supernatant. The obtained supernatant is further mixed with ethanol of 1 to 3 volumes thereof. The mixture is further centrifuged to obtain a precipitate. The precipitate obtained is dissolved in distilled water and 30 the solution obtained, is dialyzed. The dialyzate obtained is also an ingredient or ingredients which are capable of preventing the induction or metastasis of cancer of the present invention.

The agaricus extract or the ingredient or ingredients contained therein, which are capable of preventing the induction or metastasis of cancer, prepared as described
5 above can be used for production of medicines by themselves or in combination with various carriers.

Typically, the agaricus extract or the ingredient or ingredients contained therein, which are capable of
10 preventing the induction or metastasis of cancer, prepared as described above, can be formulated as a composition capable of being taken orally with a biocompatible pharmaceutical carrier (for example, physiological saline, buffered physiological saline, dextrose, water, and the like).

15 The above described pharmaceutically acceptable carriers are known to those skilled in the art and include, for example, the following carriers but not limited to these: buffers such as Ringer's solution, Hank's balanced salt
20 solution, or buffered physiological saline; fatty acids such as sesame oil; synthetic fatty acid esters such as ethyl oleate or triglycerides; saccharides such as lactose, sucrose, mannitol, sorbitol; starches derived from vegetables such as corn, wheat, rice, or potato; cellulose such as me-
25 thylcellulose, hydroxypropylmethylcellulose, or sodium carboxymethylcellulose; rubber such as gum arabic or tragacanth; proteins such as gelatin or collagen; cross-linked polyvinyl pyrrolidone, agar, alginic acid or salts thereof, or the like.

30 The agaricus extract or the ingredient or ingredients contained therein, which are capable of preventing the induction or metastasis of cancer, prepared as described

above can be ingested alone or in combination with other drugs or foodstuffs.

5 The agaricus extract, or the ingredient or ingredients
contained therein, which are capable of
preventing the induction or metastasis of cancer, and the
composition comprising them, prepared as described above,
can be administered orally or parenterally. Parenteral
administration is accomplished via intravenous, intra-
10 muscular, intra-abdominal or intranasal administration.
The details of formulation and administration of the
pharmaceutical composition according to the present in-
vention can be performed in accordance with descriptions
in a textbook in the field of art, for example, "REMINGTON'S
15 PHARMACEUTICAL SCIENCES" (Maack Publishing Co., Easton, PA).

 The agaricus extract, or ingredient or ingredients
contained therein which are capable of preventing the
induction or metastasis of cancer for oral administration
20 can be formulated as a composition including a pharma-
ceutically acceptable carrier well known in the art, in a
prescription form suitable for ingestion. Such a carrier
allows the composition obtained to be formulated as a tablet,
pill, sugar-coated pill, capsule, liquid, gel, syrup, slurry,
25 suspension, or the like, suitable for ingestion by patients.

 The composition of the present invention includes the
agaricus extract or ingredient or ingredients contained
therein in an amount effective for preventing the induction
30 or metastasis of cancer. Those skilled in the art will
thoroughly understand and recognize the "pharmacologically
effective amount for preventing the induction or metastasis
of cancer". Initially, the "pharmacologically effective

amount for preventing the induction or metastasis of cancer" can be evaluated by *in vitro* assay via cell culturing or an appropriate animal model. Then, using such information (obtained in the initial *in vitro* assay or animal model),
5 the effective amount for ingestion in human can be determined. The "pharmacologically effective amount for preventing the metastasis of cancer" can be determined, for example, in the assay using A/J mouse in which lung cancer is induced by administering NNK, as described herein.

10

The amount of the agaricus extract which is actually ingested depends on the health conditions, or the like of the individual to which the extract is applied and may be optimized so that a desirable effect can be achieved. It
15 is a routine process for those skilled in the art to determine a pharmaceutically or nutritionally effective amount.

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The agaricus extract, or an ingredient or ingredients contained therein, capable of preventing the induction or metastasis can be mixed with one or more selected foodstuffs in an amount sufficient for exerting its function. The one
25 or more selected foodstuffs are mixed with the fraction having immune activation activity in a form known to those skilled in the art, usually, powder form. The mixture can be served as a liquid food product depending on its utility or on
30 preference. Alternatively, the mixture may be formed as capsules such as hard capsules or soft capsules, tablets, or pills, or may be formed into a powdery, granular, tea-leaf, tea-bag, or candy form.

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The agaricus extract, or an ingredient or ingredients contained therein, contained therein, which are capable of preventing the induction or metastasis of cancer prepared

as described above, inhibits lung cancer induced by NNK or urethane, and the metastasis thereof. The present invention is based on studies regarding therapies by agaricus extract in various administration forms against carcinogenic substances existing in fermented food, and those in tobacco smoke. In reference to procedures, protocols or guidance disclosed herein, the effectiveness of the above described agaricus extract, or an ingredient or ingredients contained therein contained therein, which are capable of preventing the induction or metastasis of cancer, against cancer induced by other carcinogenic substances, can be easily confirmed.

That is, the effectiveness of the above described agaricus extract, or an ingredient or ingredients contained therein, capable of preventing the induction or metastasis of cancer can be easily confirmed by examining the effects on expression level of the cyclin D1 gene and expression level of PCNA (an antigen of growing cell) in lung tissue of mouse to which a carcinogenic substance has been administered, caused by foods which contain varying concentrations of agaricus extract, or an ingredient or ingredients capable of preventing the induction or metastasis of cancer.

Similarly, the effectiveness of the above described agaricus extract, or an ingredient or ingredients contained therein, capable of preventing the induction or metastasis of cancer can be easily confirmed by examining whether or not the incidence of lung cancer in mouse, to which a carcinogenic substance has been administered, is inhibited; by examining whether or not the expression level of the cyclin D1 gene in lung tissue of mouse, to which a carcinogenic substance has been administered, is suppressed; and by examining whether or not the expression level of PCNA (an

antigen of growing cells) in mouse lung tissue is suppressed, caused by foods which contain varying concentrations of agaricus extract, or an ingredient or ingredients being capable of preventing the induction or metastasis of cancer.

5

Hereinafter, the present invention will be further described by way of examples using urethane and NNK as carinogens. The following examples are merely illustrative and do not limit the present invention.

10

Examples

Hereinafter, the present invention is illustrated by the following examples.

15 (Example 1) Preparation of agaricus extract.

(1) The above described dried product A was used as a agaricus hot water extract. This product A was obtained by extracting a dried fruit body of agaricus (Kyowa's agaricus mushroom (available from Kyowa Engineering Co., Ltd.)) with
20 boiling water, removing residue by centrifugation, at 1800 x g, for 10 minutes followed by lyophilization. The product A is dissolved in purified water at a concentration of 3.7 mg/ml to form Sample I, and at a concentration of 8 mg/ml to form Sample II.

25

(2) Distilled water (2 L) was added to 300 g of Kyowa's agaricus mushroom, and the mixture was heated to reflux for two hours. The solution obtained was filtered to separate a filtrate (a solution extracted with hot water) and a residue.
30 Again, 2 L of distilled water was added to the residue and the mixture was heated to reflux for another two hours to perform hot water extraction and a filtrate was obtained. Further, the same procedure was repeated one more time. The

filtrates obtained were lyophilized together to obtain dried product A (153 g: extraction rate of 51%).

5 Distilled water (500 mL) was added to 50 g of dried
product A and the mixture was put into a dialysis tube
(Spectra/Por Membrane 50x31, inner diameter of 8 mm and length
of 30 cm, FE-0526-65). The mixture was dialyzed against 3
L of distilled water for 12 hours. The dialyzate obtained
was lyophilized to obtain dried product C (27 g: extraction
10 rate of 53%). The solution remaining in the dialysis tube
was further dialyzed against running water for 30 hours,
and then dialyzed twice against distilled water (four hours
each time, total 8 hours). Thereafter, the solution
remaining in the dialysis tube was lyophilized to obtain
15 dried product B (11 g: extraction rate of 22%). Subsequently,
3 g of dried product C was dissolved in 30 mL of distilled
water and chromatography using TOYOPEARL HW40C (inner
diameter of 40 mm and length of 420 mm) was performed. The
eluent was entirely distilled water. For each fraction, 20
20 ml of the aliquots were taken to obtain fractions 1 to 30.
These fractions were divided into the following five groups
with reference to results of thin-layer chromatography
analysis. The dried weights were as follows: fractions 1
to 11 (75 mg, 2.5%); fractions 12 to 15 (920 mg, 30.7%);
25 fractions 16 to 17 (1570 mg, 52.3%); fractions 18 to 19 (270
mg, 9%); and fractions 20 to 28 (97 mg, 3.2%).

30 Infrared radiation (IR) absorption spectrum data of
fraction 16 (hereinafter, referred to as 1SY-16) was as
follows.

Fraction 16: IR (KBr) 3390, 3325, 3285, 2940, 2920, 1641,
1634, 1622, 1615, 1600, 1595, 1405, 1394, 1084, 1020:
molecular weight (estimated by gel filtration) 100-2000 Da

(3) Hot water extraction similar to that described above was performed to obtain 6 L of a combined filtrate (a solution extracted with hot water). The filtrate was concentrated under reduced pressure to 1 L, and 1 L of ethanol was added thereto and mixed followed by centrifugation to obtain precipitate and supernatant. Ethanol (3 L) was further added to the supernatant and mixed, and the mixture was centrifuged to obtain a precipitate, and the precipitate was dissolved in distilled water and dialyzed. The external dialyzate obtained was lyophilized to obtain a powder (hereinafter referred to as ABMK-22).

(Example 2) Prevention of lung cancer induced by NNK, by means of agaricus extract.

Fifty A/J mice (male, 7 weeks age) were divided into 5 groups, which were administered with Sample I, Sample II, ABMK22, and 1SY16 described in Example 1, as well as a control group, respectively. NNK (4 mg per kg body weight) was administered via a single gavage to each mouse in each group. This amount of NNK administered to each mouse corresponds to the amount of NNK which would have been ingested during one hundred years, if a person were to smoke a box of tobacco per day (Djordijevic, M.V. et al., Comparison of selected components in the mainstream smoke of the leading U. S. and Japanese cigarettes. In: Proceedings of the CORESTA smoke and Technology Meeting, pp.200-217, November 3 to 8, 1996). Subsequently, the agaricus extract was administered to each mouse of each group as described below.

Control group: mice allowed to freely ingest normal drinking water .

Sample I group: mice allowed to freely ingest drinking water containing 30%(v/v) of Sample I for 16 weeks.

5 Sample II group: mice allowed to freely ingest drinking water containing 60%(v/v) of Sample II for 16 weeks.

The amount of water ingested by each mouse in the above described groups, was about 5ml/mouse/day, respectively.

10 ABMK22 group: ABMK22 was administered via a gavage at a dose of 100mg/kg body weight for 7 days.

15 1SY16 group: 1SY16 was administered via a gavage at a dose of 100mg/kg body weight for 7 days.

Mice in each group were sacrificed 16 weeks after administering NNK and dissected, and the incidence of lung cancer was examined using a dissection observation microscope. The results are shown in figure 1. The horizontal axis in figure 1 indicates each test group, and the vertical axis indicates the incidence of lung cancer (the morbidity of lung cancer in each group provided that the morbidity of the control group is made to be 100%).

25 As indicated in figure 1, agaricus extract reduced significantly the incidence of lung cancer as compared with the control group (the results in Sample I group and Sample II group, showed significant difference at $P < 0.05$ and $P < 0.01$, respectively, as compared with the control group. Significance for Sample I group and Sample II group is represented by * and **, respectively in figure 1), and ingredients ABMK22 and 1SY16 containing in agaricus extract further reduced the incidence of lung cancer as compared with the agaricus

extract (the results for ABMK22 group and 1SY16 group, are statistically significant at $P < 0.01$ and $P < 0.001$, respectively as compared with the control group. Significance for ABMK22 group and 1SY16 group is represented by as ** and ***, respectively in figure 1). In the event that the results are represented as the ratio of suppression (100 minus the ratio of incidence), as seen in figure 1, the ratio of suppression for Sample I, Sample II, ABMK22 and 1SY16 were about 30%, about 35%, about 50% and about 82%, respectively.

In this way, it was shown that the ingestion or administration of agaricus extract and ingredients contained therein suppressed the generation of cancer induced by NNK.

(Example 3) Prevention of lung cancer induced by NNK, by means of agaricus extract.

Control group, and three test groups, each of which was allowed to freely ingest drinking water containing 15%(v/v), 30%(v/v) or 60%(v/v) of the above described Sample II were provided, and lung cancer was induced by NNK similarly as described in Example 2. Subsequently, similar to Example 2, mice in each group were sacrificed 16 weeks after administering NNK and dissected, and the incidence of lung cancer was examined using a dissection observation microscope. The results are shown in figure 2. The horizontal axis in figure 2 indicates each test group, and the vertical axis indicates the incidence of lung cancer (the morbidity of lung cancer in each group provided that the morbidity of the control group is made to be 100%).

As indicated in figure 2, agaricus extract reduced significantly the incidence of lung cancer as compared with

the control group (the results in test groups, each of which was allowed to freely ingest drinking water containing 15%(v/v), 30%(v/v) or 60%(v/v) of Sample II, showed significant difference at $P<0.05$, $P<0.05$ and $P<0.01$, respectively as compared with the control group. They are represented by * and **, respectively in figure 2).

In this way, it was shown that the ingestion or administration of agaricus extract and ingredients contained therein suppressed the generation of cancer induced by NNK in a dose dependent manner.

(Example 4) Suppression of gene or gene product induced by administering NNK, by means of agaricus extract.

The above described Sample I, ABMK22, and 1SY16 were administered to each group of A/J mice, which were grouped similarly as in Example 2 except for not providing a group receiving Sample II. Similar to Example 2, mice in each group were sacrificed 16 weeks after administering NNK and dissected, and the expression of cyclin D1, proliferating cell nuclear antigen (PCNA), and the expression of cyclin-dependent kinase 4 (CDK4) in lung tissue was examined. Cyclin D1, PCNA, and CDK4 were measured according to the method described in I.P.Lee, Pulmonary cyclin D1-induction by Fumonisin B1 in Female A/J mice, The 91th Cancer Society: Paper No.5313, April 15, 2000.

Generally speaking, cells during growth have a cell cycle comprised of division phase (M phase) and inter phase (G1 phase, S phase and G2 phase). DNA synthesis occurs in a part of inter phase (S phase). The genetic information is replicated in S phase and is equally divided in M phase into two daughter cells.

CDK 4 is a member of the protein phosphorylating enzyme family, and controls advancement through the cell cycle. Therefore, CDK 4 is so to speak, an engine of cell cycle advancement. Cyclin D1 has a role to advance cells in G1 phase to S phase by combining with CDK4. Cyclin D1 plays a role, so to speak, as an accelerator, and as a regulating factor for CDK4. PCNA is a subunit of DNA polymerase which advances cells into S phase, and thus also corresponds to an engine for advancing the cell cycle. The aberrant overexpression of these proteins leads to the induction of cancer, and thus, genes encoding these proteins are oncogenes. It is possible to evaluate whether or not cells are in the process leading to the incidence of cancer by analyzing these gene products.

Figure 3 shows the results of measuring expression of PNCA, cyclin D1 and CDK4 in lung tissue of A/J mice in each group. Right-hand portion of figure 3 indicates bands of PCNA, cyclin D1 and CDK4 when lung samples in each group were subject to electrophoresis. Light and shade represent strength of the expression level. As indicated in the right-hand portion of figure 3, PNCA, cyclin D1 and CDK4 in the control group are expressed abnormally as dense bands were detected, as such cancer has been induced. However, in groups being administered with Sample I, ABMK22, and 1SY16, less dense bands of PCNA and cyclin D1 are detected indicating that these proteins have not expressed abnormally. The left-hand portion of figure 3 represents the quantified results in each group as a bar graph. The vertical axis represents the amount of expression provided that the amount of expression in the control group was made to be 100%, and the horizontal axis represents PCNA, cyclin D1, and CDK4

expression in each group. Four bars in each measured item represent, left to right, control group, Sample I group, ABMK22 group, and 1SY16 group. As indicated in the left-hand portion of the figure, the expression levels of PCNA and cyclin D1 in Sample I group, ABMK22 group, and 1SY16 group were less than 10% compared with the expression level in control group (statistically significant differences at $P < 0.01$).

In this way, it was shown that the ingestion or administration of agaricus extract and ingredients contained therein suppressed the expression of the oncogenes induced by NNK.

(Example 5) Suppression of gene or gene product induced by administering NNK, by means of agaricus extract.

Similar to Example 4, the expression of cyclin D1, proliferating cell nuclear antigen (PCNA), and cyclin-dependent kinase 4 (CDK4) in lung tissues was examined from mice allowed to freely ingest drinking water containing 15, 30 or 60% of Sample II.

Figure 4 shows the results of measuring expression of PNCA, cyclin D1 and CDK4 in A/J mice of each group. The right-hand portion of figure in figure 4 indicates bands of PCNA, cyclin D1 and CDK4 when lung samples in each group were subject to electrophoresis. Light and shade represent strength of the expression level. As indicated in the right-hand portion of figure 4, PNCA, cyclin D1 and CDK4 in the control group are expressed abnormally, as dense band were detected, as such cancer has been induced. However, in groups being administered with drinking water containing 15, 30 or 60% of Sample II, less dense bands of PCNA and

cyclin D1 are detected, the higher the concentration of Sample II became, which indicated that Sample II suppressed the abnormal expression of these proteins in a dose dependent manner. The left-hand portion of figure 4 represents the quantified results in each group as a bar graph. The vertical axis represents the amount of expression provided that the amount of expression in the control group was made to be 100%, and the horizontal axis represents measured PCNA, cyclin D1, and CDK4 expression in each group. Four bars in each measured item represent, left to right, control group, groups ingesting drinking water containing 15, 30% or 60% of Sample II, respectively. As indicated, Sample II was indicated to suppress the abnormal expression of these proteins in a dose dependent manner (in groups ingesting drinking water containing 15, 30% or 60% of Sample II, the expression of cyclin D1 and PCNA showed statistically significant differences at $P < 0.01$, compared with the expression of cyclin D1 or PCNA in control group)

In this way, it was shown that the ingestion or administration of agaricus extract and ingredients contained therein suppressed the expression of the oncogenes induced by NNK in a dose dependent manner.

(Example 6) Prevention of lung cancer induced by urethane, by means of agaricus extract.

Tests were conducted similar to Example 2, except for providing groups allowed to freely ingest drinking water containing 30% (v/v) Sample I, and 30% Sample II, respectively, and a group administered with ABMK22 as described in Example 2. In addition, instead of NNK, urethane was administered by a gavage in an amount about 20,000 times the exposure level in human (250 mg/kg mouse body weight).

Similar to Example 2, mice in each group were sacrificed 16 weeks after administering urethane and dissected, and the incidence of lung cancer was examined using a dissection observation microscope. The results are shown in figure 5. The horizontal axis in figure 5 indicates each group tested, and the vertical axis indicates the incidence of lung cancer (the morbidity of lung cancer in each group provided that the morbidity of the control group is made to be 100%).

As indicated in figure 5, agaricus extract reduced significantly the incidence of lung cancer as compared with the control group (the results in test groups, showed statistically significant differences in Sample I group at $P < 0.05$, and in Sample II group at $P < 0.01$, as compared with the control group. They are represented by * and **, respectively in figure 5). ABMK22 contained in agaricus extract further reduced the incidence of lung cancer, compared with agaricus extract (the result showed statistically significant differences at $P < 0.001$, as compared with the control group. Significance is represented by *** in figure 5). In the event that the results are represented as the ratio of suppression (100 minus the ratio of incidence), as seen in figure 5, the ratio of suppression for Sample I, Sample II, and ABMK22 was about 30%, about 60%, and about 82%, respectively.

In this way, it was shown that the ingestion or administration of agaricus extract and ingredients contained therein suppressed the generation of cancer induced by urethane.

(Example 7) Prevention of genes or gene products induced

by urethane, by means of agaricus extract.

Tests were conducted similar to Example 2, except for providing groups allowed to freely ingest drinking waters containing 30%(v/v) Sample I, and 30% Sample II, respectively, and a group administered with ABMK22 as described in Example 2. In addition, as well as instead of NNK, urethane was administered by a gavage in an amount about 20,000 times the exposure level in human (250 mg/kg mouse body weight).

Similar to Example 4, the expression of cyclin D1, proliferating cell nuclear antigen (PCNA), and cyclin-dependent kinase 4 (CDK4) in lung tissues of each group was examined.

Figure 6 shows the results of measuring expression of PNCA, cyclin D1 and CDK4 in A/J mice of each group. The right-hand portion of figure 6 indicates bands of PCNA, cyclin D1 and CDK4 when lung samples in each group were subject to electrophoresis. Light and shade represent strength of the expression level. As indicated in the right-hand portion of figure 6, PNCA, cyclin D1 and CDK4 in the control group are expressed abnormally, as dense bands were detected, as such these proteins were expressed abnormally at a high level, and the cancer has been induced. However, in groups allowed to ingest drinking water containing Sample I and Sample II, and the group administered with ABMK22, each band was less dense compared with the control group, indicating that abnormal expression of each gene has been suppressed. The left-hand portion of figure 6 represents the quantified results in each group as a bar graph. The vertical axis represents the amount of expression, provided that the amount expression in the control group was made to be 100%, and the horizontal axis represents measured PCNA, cyclin D1,

and CDK4 expression in each group. Four bars in each measured item represent, left to right, control group, groups ingesting drinking water containing Sample I, and Sample II, and a group administered with ABMK22. As indicated in the figure, especially ABMK22 was indicated to suppress the abnormal expressions of these genes (statistically significant differences at $P < 0.05$).

In this way, it was shown that the ingestion or administration of agaricus extract suppressed the expression of the oncogenes induced by urethane.

(Example 8) Prevention of rat colon cancer induced by AOM, by means of agaricus extract.

It has been known that azoxymethane (AOM) is a carcinogenic substance which induces large intestine cancer and forms aberrant crypt foci (ACF) (Carcinogenesis 21(6):1149-1155, 2000). β -catenin gene often has been mutated in colon cancer in mouse and rat induced by AOM (Takahashi M. et.al., Carcinogenesis, 21:1117-1120, 2000). In aberrant dysplasia ACF, both of modified intracellular localization of β -catenin and increased expression of inducible type nitrogen mono oxide synthetase (iNOS) were detected, but not detected in hyperplasia ACF (Takahashi M. et.al., Carcinogenesis, 21:1319-1327, 2000). Accordingly, ACF in mouse and rat induced by AOM can be used as an assay system for evaluating the process in which cancer is being induced, and the process in which cancer is metastasizing.

By using ACF induced by AOM as an assay system, it was evaluated whether or not an ingredient or ingredients contained in agaricus extract prevents the induction of

cancer.

Thirty rats (female, 8 to 9 weeks age) were divided into 3 groups with each group consisting of 10 rats, which were the 1SY16 administered groups (120mg/kg body weight group and 60mg/kg body weight group), and the control group. To each rat of each group, 15 mg/kg body weight of AOM was injected twice a week subcutaneously.

Subsequently, rats of each group, were administered agaricus extracts as indicated below. 1SY16 was dissolved in a solution of 0.1% carboxymethylcellulose, and administered in a dose of 0.5% of body weight. Administration occurred twice a week, and continued for four weeks.

120 1SY16 group: 1SY16 was administered by a gavage in a dose of 120 mg/kg body weight.

60 1SY16 group: 1SY16 was administered by a gavage in a dose of 60 mg/kg body weight.

Control group: carboxymethylcellulose was administered by a gavage in a dose of 0.5% of body weight.

Mice in each group were sacrificed 13 weeks after administering AOM and dissected, and the numbers of ACF were counted using a dissection observation microscope. The results are shown in figure 7. The horizontal axis in figure 7 indicates each group tested, and the vertical axis indicates the average numbers of generated ACF per mouse.

As indicated in figure 7, 1SY16 reduced the numbers of generated ACF significantly compared with the control group

(significant differences at $P < 0.05$, indicated by * in figure 7). In the event that they are represented as the ratio of suppression, it is confirmed that the incidence of large intestine cancer in groups administered with 1SY16 of 60 mg/kg body weight and 120 mg/kg body weight were suppressed by about 72% and 82.5%, respectively, compared with the control group, indicating that the ingestion or administration of 1SY16 suppressed the generation of cancer induced by AOM.

As indicated above, since the ingestion or administration of agaricus extract or ingredients contained therein suppressed the expressions of a gene or gene product increased by the administrations of NNK and urethane in a dose dependent manner, it is considered that an ingredient or ingredients which suppress the carcinogenic conditions induced by the carcinogenic substance are contained in agaricus extract.

INDUSTRIAL APPLICABILITY

Material for drugs and foodstuffs which prevents the induction and metastasis of cancer potentially are provided. More specifically, material for drugs and foodstuffs which prevents the incidence of lung cancer by carcinogenic substances targeting lung, and the metastasis which occurs as a result thereof are provided.